Poster board # E209

The long noncoding RNA *uc.230*/CUG-binding protein 1 axis sustains intestinal epithelial homeostasis and response to tissue injury

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ABSTRACT

Long noncoding RNAs transcribed from ultraconserved regions (T-UCRs) control different cell functions and are involved in gut mucosal pathologies. Here, we investigated the role of T-UCRs in intestinal epithelium and identified T-UCR *uc.230* as a major regulator of epithelial homeostasis. METHODS: Intestinal mucosal tissues were collected from mice treated with 3% DSS in drinking water and from patients with ulcerative colitis and Crohn's disease. Levels of *uc.230* were silenced in intestinal epithelial cells (IECs) and organoids by transfection with small interfering RNAs or elevated using a plasmid vector that overexpressed *uc.230*. Association of *uc.230* with miR-503 was determined by biotinylated RNA pulldown assays. RESULTS: Compared with controls, intestinal mucosal tissues from mice with DSS-induced colitis and from patients with ulcerative colitis had increased levels of *uc.230*. Silencing *uc.230* inhibited the growth of IECs and intestinal organoids and resulted in epithelial barrier dysfunction. Silencing *uc.230* also increased IEC vulnerability to apoptotic cell death, whereas increasing *uc.230* levels protected IECs against apoptosis. Mechanistic studies revealed that *uc.230* increased CUG-binding protein 1 (CUGBP1) by acting as a natural decoy RNA for miR-503, which interacts with *Cugbp1* mRNA and represses its translation. CONCLUSIONS: Our results indicate that *uc.230* sustains intestinal mucosal homeostasis by promoting epithelial renewal and barrier function and protects IECs against apoptosis at least in part by serving as a natural sponge for miR-503, thereby preserving CUGBP1 expression.

INTRODUCTION

The mammalian intestinal epithelium is colonized by complex microbiota and exposed to a wide variety of luminal noxious substances. Intestinal epithelial integrity is commonly disrupted in patients with critical disorders, but the exact underlying mechanisms are unclear.

LncRNAs regulate a variety of cellular processes and are intimately involved in diverse human diseases by working jointly with miRNAs, RNA-binding proteins (RBPs). RNAs transcribed from ultraconserved regions (T-UCRs) represent a class of novel endogenous lncRNAs that are implicated in many cellular functions and human diseases.

We recently reported that 21 T-UCRs, including *uc.230*, are differentially expressed in the intestinal mucosa responding to stress, but the function of most these T-UCRs in the intestinal epithelium homeostasis remains unknown. This study provides evidence showing that *uc.230* is a regulator of epithelial renewal, apoptosis, and barrier function via interaction CUGBP1.

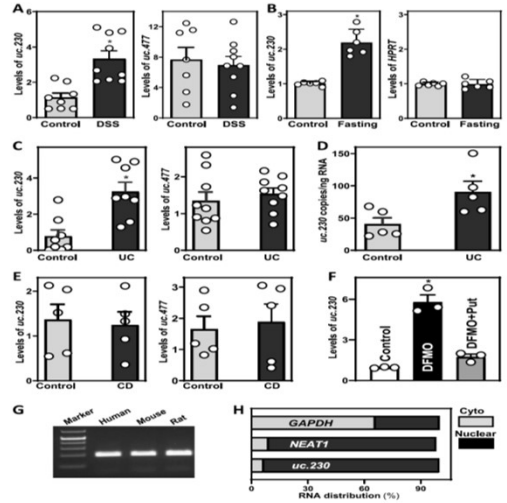

Figure 1. Changes in expression of *uc.230* in the intestinal mucosa in various pathologies. (*A*) Levels of mucosal *uc.230* and *uc.477* in the colon in mice treated with water (control) or 3% DSS for 5 days. (*B*) Levels of mucosal *uc.230* in the small intestine in mice fasted for 48 hours. (*C,D*) Total levels and quantification of copy numbers of *uc.230* in the colonic mucosa from patients with active ulcerative colitis (UC). (*E*) Levels of tissue *uc.230* and *uc.477* in the ileal mucosa in patients with Crohn's disease (CD). (*F*) Levels of *uc.230* in cultured Caco-2 cells exposed to DFMO alone or DFMO plus putrescine (Put) for 6 days. (*G*) Validation of *uc.230* transcripts (238 bp) in three different genomes. (*H*) Levels of cytoplasmic (cyto) and nuclear *uc.230*, lncRNA *NEAT1*, and *Gapdh* mRNA in Caco-2 cells.

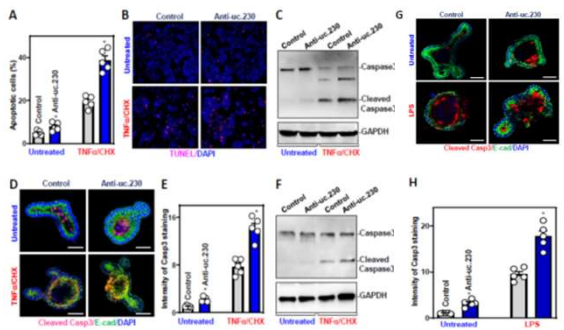

Figure 3. *uc.230* silencing increases apoptosis. (*A*) Percentages of apoptotic cells after *uc.230* silencing. (*B*) Images of TUNEL staining in cells described in *A*. Original magnification ×150. (C) Changes in levels of caspase-3 in cells described in *A*. (*D*) Cleaved caspase 3 staining in intestinal organoids after *uc.230* silencing. Intestinal organoids transfected with anti-uc.230 or control oligo (control). Forty-eight hours after transfection. (*E*) Quantitative data of cleaved caspase 3 staining in organoids described in *D*. (*F*) Changes in levels of cleaved caspase-3 in intestinal organoids described in *D*. (*G,H*) Apoptotic cell death in organoids exposed to LPS after *uc.230* silencing.

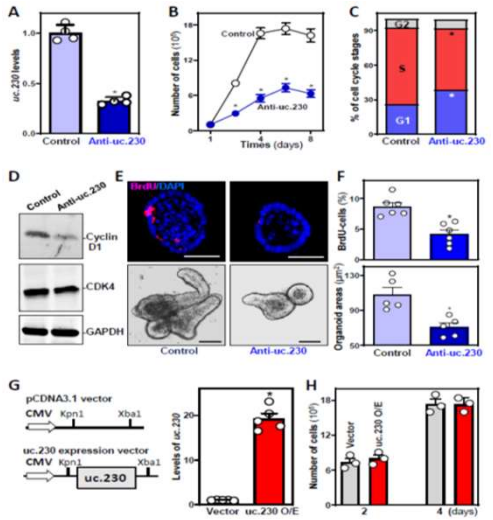

Figure 2. *uc.230* is required for renewal of the intestinal epithelium. (*A*) Levels of *uc.230* in Caco-2 cells after transfection with anti-uc.230 or control oligo (control). (*B*) Cell growth after *uc.230* silencing *in vitro*. (*C*) The relative G1, S, and G2/M compartments of cell cycle after anti-uc.230 transfection. (*D*) Levels of cyclin D1 and CDK4 in cells described in *C*. (*E*) Growth of intestinal organoids after *uc.230* silencing *ex vivo*. (*F*) Quantification of BrdU and surface of intestinal organoids described in *E*. (*G*) Levels of *uc.230* in cells transfected with the *uc.230* expression vector. (*H*) Cell growth on days 2 and 4 after *uc.230* overexpression.

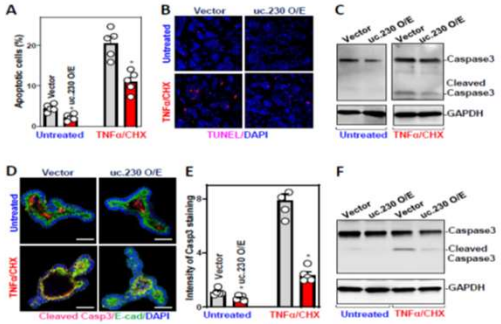

Figure 4. *uc.230* protects the intestinal epithelium against apoptosis. (*A*) Percentages of apoptotic cells after *uc.230* overexpression. (*B*) Images of TUNEL staining in cells described in *A*. (*C*) Levels of cleaved caspase-3 in cells described in *A*. (*D*) Cleaved caspase 3 staining in intestinal organoids after *uc.230* overexpression. Forty-eight hours after transfection with *uc.230* expression vector or control vector, organoids were exposed to TNFα/CHX, and apoptotic cell death was examined 3 h thereafter. Scale bars: 100 μm. (*E*) Quantitative data of cleaved caspase 3 staining in organoids described in *D*. Values are means ± SEM. * $P < 0.05$ compared with control vector. (*F*) Changes in levels of cleaved caspase-3 in intestinal organoids treated as described in *D*.

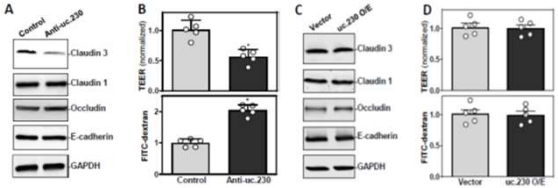

Figure 5. *uc.230* is essential for intestinal epithelial barrier function. (*A*) Representative immunoblots of tight junctions and adherens junction after *uc.230* silencing. (*B*) Disrupted barrier function as indicated by changes in TEER (*top*) and FITC-dextran paracellular permeability (*bottom*). (*C,D*) Effect of ectopically overexpressed (O/E) *uc.230* on epithelial barrier function.

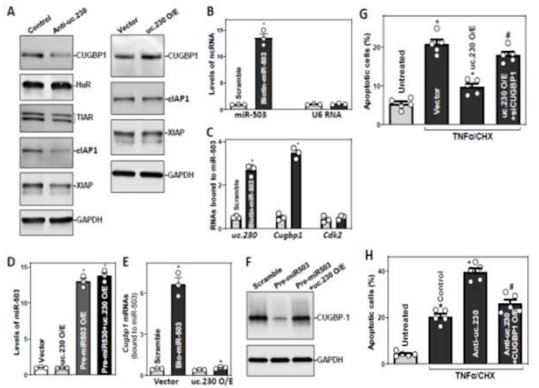

Figure 6. *uc.230* prevents apoptosis by increasing CUGBP1 via interaction with miR-503. (*A*) Immunoblots of RBPs and anti-apoptotic proteins after silencing or overexpressing *uc.230*. (*B*) Levels of biotinylated miR-miR-503 (left) and *U6* RNA (right) 24 h after transfection with biotinylated miR-503. (*C*) Binding of biotinylated miR-503 to *uc.230* and mRNAs *Cugbp1* and *Cdk2*. (*D*) Levels of miR-503 48 h after cells were transfected with pre-miR-503 alone or co-transfected with pre-miR-503 and *uc.230* expression vector. (*E*) Effect of increasing *uc.230* on *miR-503/Cugbp1* mRNA association. (F) Representative immunoblots of CUGBP1in cells transfected with pre-miR-503 alone or co-transfected with pre-miR-503 and *uc.230* expression vector. (*G,H*) Percentages of TNFα/CHX-induced apoptosis in cells co-transfected with *uc.230* expression vector and siCUGBP1 or co-transfected with *anti-uc.230* and CUGBP1 expression vector.

CONCLUSIONS

1.uc.230 sustains the gut epithelial homeostasis by altering proliferation, apoptosis, and barrier function.

2.uc.230 increases CUGBP1 expression by inhibiting association of *Cugbp1* mRNA with miR-503.

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